(18) Measurement of Neutrophil Elastase Activity with 
(N-Benzoyloxycarbonyl-Ala-Ala)\textsubscript{2}-Rhodamine 110

Submitted by:  
Gregor Rothe and Günter Valet  
Arbeitsgruppe Zellbiochemie  
Max-Planck-Institut für Biochemie  
W-8033 Martinsried  
Germany

Phone: 49-(89)-8578-2525  
Fax: 49-(89)-8578-3777

Outline

Elastase is a lysosomal serine proteinase contained in large amounts in neutrophils, in lower amounts in monocytes, which degrades a large variety of biological substrates at neutral pH. Elastase is important for the degradation of bacteria. The extracellular release of elastase may lead to inflammatory tissue destruction in diseases such as sepsis, posttraumatic shock, myocardial reperfusion, or rheumatoid diseases. The nonfluorescent (N-benzoyloxycarbonyl-Ala-Ala)\textsubscript{2}-rhodamine 110 is intracellularly cleaved by elastase in a sequential way to the green fluorescent N-benzoyloxycarbonyl-Ala-Ala-rhodamine 110 and free rhodamine 110. The specificity of the cellular fluorescence for elastase activity is shown by inhibition with the serine proteinase inhibitor DFP.

Specimen: 3 ml heparinized human blood (10 U heparin/ml)

Reagents

HBSS without phenol red or bicarbonate, supplemented with 10 mM HEPES (pH 7.35)
(N-benzoyloxycarbonyl-Ala-Ala)\textsubscript{2}-rhodamine 110 (MW 1028.79)  
stock solution: 4 mM in DMP (4.12 mg/ml)
propidium iodide (PI) (MW 668.4)  
stock solution: 3 mM (2 mg/ml) in 5 mM HEPES-buffered saline (0.15 M NaCl, pH 7.35)
specific inhibitor  
diisopropylphosphofluoridate (DFP) (MW 184.15): 1 M in DMSO

Procedure

1. Layer 3 ml heparinized blood carefully on top of 3 ml lymphocyte separation medium. Allow erythrocytes to sediment for 40 minutes at room temperature.
2. Withdraw the upper 800 µl supernatant plasma and store on ice. This will contain platelets and approximately 2 x 10⁹/ml unseparated leukocytes.
3. For lysosomal elastase activity, put 1.00 ml HBSS, 20 µl cell suspension, and 1 µl (N-benzoyloxycarbonyl-Ala-Ala)\textsubscript{2}-rhodamine 110 solution in a 12 x 75 mm polypropylene test tube (final (N-benzoyloxycarbonyl-Ala-Ala)\textsubscript{2}-rhodamine,110 concentration 4 µM). Incubate at 37°C. Take 250 µl aliquots at 10, 20, and 30 minutes.
4. For specific inhibition of elastase activity, put 1.00 ml HBSS, 20 µl cell suspension, and 1 µl DFP stock in a 12 x 75 mm polypropylene test tube. Cap the tube to avoid inhalation of DFP. Incubate for 10 minutes at 37°C. Add 1 µl (N-benzoyloxycarbonyl-Ala-Ala)\textsubscript{2}-rhodamine 110 solution. Continue incubation, taking 250 µl aliquots at 10, 20, and 30 minutes after addition.
5. Counterstain dead cells by incubating 250 µl stained cell suspension with 5 µl 3 mM PI for 3 minutes on ice (final PI concentration 60 µM).
6. Run on flow cytometer. Afterwards, inactivate DFP-contaminated samples by transferral into 2 N NaOH.

Excitation: 488 nm (argon laser) or high pressure mercury arc lamp with 470-500 nm bandpass filter  
Filters: 510-530 bandpass for rhodamine green fluorescence  
600 nm longpass for PI (dead cells) red fluorescence
Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Supplier</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>Sigma Chemical Co.</td>
<td># H-1387</td>
</tr>
<tr>
<td>Propidium iodide</td>
<td>Serva</td>
<td># 33671</td>
</tr>
<tr>
<td>Diisopropylphosphofluoridate</td>
<td>Aldrich Chemical Co.</td>
<td># D12,600-4</td>
</tr>
<tr>
<td>(N-Benzylxycarbonyl-Ala-Ala)_2-rhodamine 110</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References


NOTES:
HANDBOOK OF FLOW CYTOMETRY METHODS

J. Paul Robinson, Editor

Associate Editors:
Zbigniew Darzynkiewicz,
Phillip Dean, Lynn Dressler,
Hans Tanke, and Leon Wheeless
Address all Inquiries to the Publisher
Wiley-Liss, Inc., 605 Third Avenue, New York, NY 10158-0012

Copyright © 1993 Wiley-Liss, Inc.

Printed in the United States of America.

Under the conditions stated below the owner of copyright for this book hereby grants permission to users to make photocopy reproductions of any part or all of its contents for personal or internal or organizational use, or for personal or internal use of specific clients. This consent is given on the condition that the copier pay the stated per-copy fee through the Copyright Clearance Center, Incorporated, 27 Congress Street, Salem, MA 01970, as listed in the most current issue of “Permissions to Photocopy” (Publisher’s Fee List, distributed by CCC, Inc.), for copying beyond that permitted by sections 107 or 108 of the US Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale.

While the authors, editors, and publisher believe that drug selection and dosage and the specification and usage of equipment and devices, as set forth in this book, are in accord with current recommendations and practice at the time of publication, they accept no legal responsibility for any errors or omissions, and make no warranty, express or implied, with respect to material contained herein. In view of ongoing research, equipment modifications, changes in governmental regulations and the constant flow of information relating to drug therapy, drug reactions, and the use of equipment and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each drug, piece of equipment, or device for, among other things, any changes in the instructions or indication of dosage or usage and for added warnings and precautions.

The publication of this volume was facilitated by the authors and editors, who submitted the text in a form suitable for direct reproduction without subsequent editing or proofreading by the publisher.

Library of Congress Cataloging-in-Publication Data
Handbook of flow cytometry methods / editor, J. Paul Robinson ;
associate editors, Zbigniew Darzynkiewicz ... [et al.].
p. cm.
Includes bibliographical references (p. ) and index.
QH585.5.F56H36 1993 574.87028—dc20 92-47082
CIP

The text of this book is printed on acid-free paper.